84 Azithromycin resistance in *Prevotella* species isolated from CF patients

L. Sherrard[1], K.A. Graham[1], S.J. McGrath[1], L. McIlreavey[1], J. Hatch[2], M. Muhlebach[2], D.F. Gilpin[1], J.S. Elborn[1], T. Schneiders[1], M. Tunney[1]. [1]*Queen's University, CF & Airways Microbiology Group, Belfast, United Kingdom;* [2]*University of North Carolina at Chapel Hill, North Carolina, United States*

Objective: The use of chronic azithromycin treatment has been linked with increased macrolide resistance. Although, *Prevotella* spp. are one of the most common anaerobes detected in CF pulmonary samples, the effect of chronic azithromycin exposure on this genus is unknown. The aims of this study were to

i. investigate if resistance is associated with azithromycin prescription
ii. compare azithromycin and clindamycin MICs between *Prevotella* isolates cultured from CF patients and healthy control subjects.

Methods: Isolates were grouped according to source and patient prescription of azithromycin: CF (currently prescribed), n = 27; CF (not currently prescribed), n = 30; healthy controls (none), n = 17. Susceptibility was determined by Etest® and MICs compared between groups using the Mann-Whitney test.

Results: CF isolates had significantly higher azithromycin and clindamycin MICs compared to the healthy control isolates ($P < 0.001$). Current prescription of azithromycin was associated with significantly higher azithromycin MICs ($P = 0.016$) in the CF isolates. CF isolates (not currently prescribed) had significantly higher azithromycin MICs ($P = 0.009$) compared to isolates from healthy control subjects. Isolates from the 2 CF groups had equal resistance to clindamycin ($P = 0.228$).

Conclusions: CF patients harbour isolates with increased resistance to azithromycin and clindamycin. Azithromycin resistance is associated with current azithromycin prescription. Work supported by a Department of Employment and Learning, NI (DEL) studentship to L. Sherrard and by HSC Research and Development, Public Health Agency, NI and the Medical Research Council through a US-Ireland Partnership Grant.

85 Investigation into the antibiotic resistome of the *Streptococcus* genus from adult cystic fibrosis patients

C.S. Thornton[1], M.E. Grinwis[1], C.D. Sibley[1], H.R. Rabin[2], M.G. Surette[3]. [1]*University of Calgary, Microbiology & Infectious Diseases, Calgary, Canada;* [2]*University of Calgary, Adult Cystic Fibrosis Clinic, Calgary, Canada;* [3]*McMaster University, Medicine and Biochemistry and Biomedical Sciences, Hamilton, Canada*

Objectives: The lower airways of CF patients are colonized by polymicrobial communities in which streptococci are prominent and are implicated in driving exacerbations. Multiple streptococci are present in sputum, including several novel species. Given their prominence in the CF airway microbiome and the capacity for horizontal gene transfer, our objectives were to determine rates of antibiotic resistance and molecular mechanisms of macrolide resistance.

Methods: 459 streptococcal isolates from 68 adult CF patients comprising of 16 novel and typed species underwent susceptibility testing for nine antibiotics used in CF management. Molecular mechanisms of resistance for the macrolides were determined by a PCR screen and sequencing.

Conclusion: 84% of isolates had multi-drug resistance to 3+ antibiotics. Resistance was greatest for macrolides at 51.6% (erythromycin) and 56.4% (azithromycin), but with novel isolates at 80%. The most common mechanisms of macrolide resistance acquired by horizontal gene transfer, the *mef* (efflux pump) and *erm* (target site methylation) accounted for only 53% of resistant isolates. Significantly, 23S ribosomal point mutations accounted for 47% of resistant isolates. This is not thought to be a common mechanism of macrolide resistance in the streptococci. The prevalence, species distribution and influence of therapy on resistance profiles suggest complex ecological interactions among the streptococci in CF airways with mutation, rather than only horizontal gene transfer, contributing to acquired antibiotic resistance in this community. This is in contrast to previous studies and may reflect differences in strain isolation or patient populations.

86 Real time genome sequencing to decipher the molecular mechanism of resistance of *Chryseobacterium oranimense*, a new multidrug resistant species isolated from a cystic fibrosis patient

P. Sharma[1], S. Diene[1], S.K. Gupta[1], C. Robert[1], M. Reynaud-Gaubert[1], J.-C. Dubus[1], J.-M. Rolain[1]. [1]*URMITE CNRS Aix Marseille University, Marseille, France*

Objective: The objective was to sequence the genome of *Chryseobacterium oranimense*, a multidrug resistant gram negative, yellow-pigmented bacterium from a cystic fibrosis patient in France.

Methods: Antibiotic susceptibility test was done using disc diffusion method and Etest assays. Whole genome sequencing was performed for the strain using PGM technology and sequences obtained were used to identify all antibiotic resistance (AR) determinants.

Results: The genome size is 4.45 bp. The G+C content is 37.7% assembled into 15 contigs. The MIC for imipenem and colistin was 12 μg/ml and 24 μg/ml, respectively. Whole resistome analysis shows the presence of an IND-4 like carbapenemase, as well as macrolide, tetracycline, rifampin, sulphonamide and fluoroquinolone AR genes. We found operons for Lipid A modifications and exopolysaccharide synthesis which could explain the resistance to colistin. We also found an operon for polyketide synthase likely involved in the biosynthesis of a macrolide compound and a zeaxanthin operon explaining the yellow pigmentation of this strain.

Conclusions: Whole genome sequencing provides insights into the molecular mechanism responsible for phenotypic properties of this multidrug resistant bacterium. The polyketide synthase operon found in the genome is reported for the first time and antimicrobial activity against a panel of bacterial strains is under investigation. *Chryseobacterium* species have been reported from environmental sources such as plants, vegetables or aquatic habitats and therefore may represent the source of transmission of this bacterium to humans.

87 Development of microspheres for pulmonary administration in cystic fibrosis lung disease

M. Gaspar[1], W. Couet[2,3], J.-C. Olivier[2,3], A. Pais[4], J. Sousa[1]. [1]*University of Coimbra, Faculty of Pharmacy, Center for Pharmaceutical Studies (CEF), Pharmaceutical Technology, Coimbra, Portugal;* [2]*INSERM, U 1070, Poitiers, France;* [3]*University of Poitiers, School of Medicine and Pharmacy, Poitiers, France;* [4]*University of Coimbra, Chemistry Department, Coimbra, Portugal*

Objectives: Cystic Fibrosis (CF) is a complex inherited disease, affecting particularly the respiratory system. The objective of this work is the development of drug microspheres directed at the treatment of *Pseudomonas aeruginosa,* which is the main cause of chronic airway infection in CF. These microspheres may target specific regions of the respiratory tract, reducing systemic toxicity and the frequency of drug administration, and increasing patients' compliance. Possessing the appropriate physical properties, they facilitate the dispersion of therapeutic agents in the inhaled air and reduce the deposition in the oropharynx and may modulate release kinetics.

Methods: Levofloxacin or ciprofloxacin loaded-microspheres prepared with low molecular weight chitosan were obtained by the spray drying technique. Particle size was determined by laser light diffraction, and optimised on the basis of some formulation and operational variables, resorting to experimental design. The analysis was complemented with optical, fluorescence and scanning electron microscopy (SEM), which provided additional insight on agglomerate formation and morphology. Aerodynamic studies have also been performed with an impactor, in order to evaluate lung distribution.

Conclusion: The prepared chitosan microspheres present an average diameter within the optimal interval for pulmonary administration (1–5 μm), and are spherical with a smooth surface. Higher polymer concentrations promote larger diameters and higher values of drug loading, while temperature and airflow are less relevant for the final properties of the microspheres.